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Microflora of Four Fermented Fish Sauces

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Thirty-nine microorganisms representing 11 species of bacteria, one yeast, and three filamentous fungi were isolated and identified from four fermented fish sauces: nampla, patis, koami, and ounago.

Fermented fish sauces, including some made with crustaceans, comprise a significant portion of the dietary protein consumed in Southeast Asia (1). The potential for improving the nutritional quality and shortening the processing time necessary to produce these sauces could be realized by developing controlled microbial fermentations using pure cultures of the appropriate microorganisms. To identify what these are, it would be necessary to survey the microflora of different fish sauces and determine microbial successions. The purpose of this study was to isolate and identify the microorganisms present in four different types of fish sauces at various stages of fermentation.

Fish sauces are generally prepared by mixing 3 parts of fish or crustacean with 1 part salt and allowing a natural fermentation to proceed for a period of from several months up to a year or longer. The liquid supernatant is then filtered and cured for 3 or more months before being used or bottled. Descriptions of some fish sauce processes have been published by Rao (6), Mackie et al. (4), and Saisithi et al. (9), but the specific microorganisms involved in these fermentations have received little attention. In the qualitative study reported here, 39 microorganisms representing 11 species of bacteria, one yeast, and three filamentous fungi were isolated and identified from four sauces: nampla, patis, koami, and ounago.

Representative samples of each product were inoculated directly (ca. 0.1 ml without dilution) onto replicate plates of marine agar (Difco), potato dextrose agar (BBL) containing 0.5% yeast extract, malt agar containing 10% malt extract, and brain heart infusion (BHI) agar (BBL), BHI + 5% NaCl, BHI + 10% NaCl, and BHI + 20% NaCl. Duplicate sets of plates were incubated aerobically in humidified incubators and anaerobically in Gas-Pak jars (BBL) containing hydrogen + carbon dioxide gas generator packs. Potato dextrose agar isolation plates were incubated at 25 C for 7 days to encourage the growth of yeasts and filamentous fungi; all other media were incubated at 30 C for 14 days. All morphologically distinct colonies were examined microscopically and pure cultures were isolated for identification. Gram stain reactions, catalase production, growth in broths containing several different concentrations of NaCl, ability to hydrolyze starch, casein, and gelatin, and to utilize glucose were determined for all isolates. Additional diagnostic tests were performed with specific groups, as necessary. Details of the methods used were described previously (10). Bacterial isolates were identified following *Bergey's Manual of Determinative Bacteriology* (3), yeasts following the methods of van der Walt (12), and fungi following keys provided in appropriate references (7, 8, 13).

Nampla is traditionally prepared in Thailand from small pelagic fishes (*Stolephorus* and *Sardinella* spp.) which are caught using purse seines. The catch is rarely refrigerated and 24 to 48 h may elapse before the fish are processed. In a standard fermentation, nampla is prepared by mixing 2 parts of fish with 1 part of marine salt and allowing the mixture to stand overnight. The fish are then placed in a large vat and covered with concentrated brine. Fermentation is allowed to proceed for 10 months if incubated in the sun or 12 months if in the shade. When the fermentation is completed, the sauce is filtered through a cloth into a large jar. This constitutes first grade nampla. The sauce may be ripened further in earthenware containers for 1 to 3 months. Fresh brine may be added to the residue to allow a second fermentation to occur for several weeks. The product of this second fermentation is second grade nampla. The finished products are clear dark brown liquids with a characteristic "salted meat" aroma and flavor.

Three samples of first grade nampla, collected after 1, 7, and 12 months of fermentation, and two samples of second grade nampla, col-

lected after 1 day and 1 month of fermentation, were supplied by the Tang Tai Chieng and Pirode Nampla plants near Bangkok, Thailand.

All of the isolates obtained from the nampla were species of *Bacillus* (Table 1). Yeasts, fungi, and obligately anaerobic bacteria were not found.

Patis, commonly produced in the Philippines, is prepared by mixing 3 or 4 parts small fish (*Stolephorus* spp., *Sardinella* spp., *Leignathus* spp., or *Decapterus macrosoma*) with 1 part marine salt (wt/wt). This mixture is placed in earthenware pots, kerosene cans, oil barrels, or large wooden vats and left to ferment at ambient temperature for 1 to 15 months. At the end of the desired fermentation period, the proteinaceous liquid patis is decanted and strained before being used directly or bottled. Some producers cook the patis before bottling. The patis residue may be ground and used as a thick sauce called bagoong. A second extraction of the residue can be made using fresh brine and a fermentation of several weeks. Although additional extractions can be made, the protein content of the resulting products is negligible (2).

Samples of patis liquid collected after 1 month of fermentation and of patis residue from a finished fermentation were obtained from the Rufina Co., Malabon, the Philippines.

The patis liquid contained single strains of *Bacillus pumilus*, *Micrococcus colpogenes*, *M. varians*, and *Candida clausenii*. This was the only fish sauce studied that contained cocci or a yeast. The patis residue contained single strains of *B. coagulans*, *B. licheniformis*, *Achromobacter thalassius*, and a strain of *B. pumilus* that differed metabolically from the strain found in the patis liquid. Fungi and obligately anaerobic bacteria were absent.

Our samples of koami and ounago were prepared from shrimp (*Mysis* spp.) and a small unidentified fish, respectively. Both products were prepared in Japan and the samples were obtained from M. Okada, Tokai Regional Fisheries Research Station, Tokyo. The koami sample was 2 years old and contained partially digested shrimp about 8 to 10 mm long immersed in a golden brown liquid having an aroma resembling salted meat. The ounago sample was 4 years old and did not contain recognizable portions of fish. The slightly viscous sauce resembled molasses in color and had an aroma similar to that of nampla. The method of preparing these sauces has not been documented but may be similar to that used for producing the Japanese sardine sauce, uwoshoyu. This sauce is made by mixing 3 parts fish with 1 part salt and allowing a natural fermentation to take place for 6 months (1).

TABLE 1. *Species of Bacillus isolated from two grades of nampla*

Species	1st Grade			2nd Grade	
	1 mo	7.mo	Final	1 day	1 mo
Bacillus cereus I[a]	−	+	−	−	−
B. cereus II	−	−	−	−	+
B. circulans	−	−	−	+	−
B. licheniformis I	+	−	+	+	−
B. licheniformis II	−	+	−	−	−
B. megaterium	−	−	+	−	−
B. pumilus	−	−	−	−	+
B. subtilis	−	−	+	−	−

[a] Roman numerals indicate variant subgroups of the same species.

The koami contained one strain each of *Bacillus cereus* and *B. sphaericus*, four strains of *B. megaterium*, and a strain of *Penicillium notatum*. The ounago contained a strain of *B. cereus* different from the one isolated from koami, *B. megaterium* identical to one of the strains isolated from the koami, and two fungi, *Cladosporium herbarum* and a member of the *Aspergillus fumigatus* group.

The fact that fermented fish sauces may contain 20 to 30% NaCl suggests that they could have a distinct halophilic microflora. In this study, isolates were not obtained from any samples plated on BHI + 20% NaCl. The few isolates which grew on BHI + 10% NaCl grew equally well or better on BHI, BHI + 5% NaCl, or marine agar which contains the osmotic equivalent of approximately 3% NaCl. Based on the criterion that true halophiles can grow on media containing 12 to 20% NaCl (5, 11), our isolates which grew on 10% NaCl appear to be halotolerant rather than halophilic. The absence of halophiles noted in these products is not unusual, however, since Nagao and Kimura (5) could not isolate halophilic microorganisms from ikashiokara, a fermented fish product containing 20% NaCl.

The results of this study indicate that species of *Bacillus* predominate the microflora of three of the fermented fish sauces studied. The predominance of sporeforming bacteria in the completely fermented products may reflect the resistant nature of these microorganisms. However, the presence of *B. pumilus* in the early stages of the patis fermentation and *B. licheniformis* during all stages of the nampla fermentation suggests that sporeforming bacilli

may play an active role early in the fermentation process.

The occurrence of *M. colpogenes* and *M. varians* in the 1-month patis indicates the possible involvement of nonsporeforming microorganisms in the early stages of some fish sauce fermentations but such organisms were not present in the 1-month nampla sample.

With the exception of the *C. clausenii* in the patis liquid, yeasts were not present in these samples of fermented sauces.

The samples of koami and ounago represented products which had undergone extensive aging of 2 and 4 years, respectively. The presence of *B. cereus* and *B. megaterium* in both products may suggest contamination from external sources, but it should be noted that these organisms were isolated from one or more of the other sauces still undergoing fermentation and from other fermented seafoods as well (10).

Since examination of the koami and ounago failed to show the presence of mycelium or other evidence of active fungal growth, the isolation of fungi from these samples was probably due to contamination with spores during the aging process.

Additional studies are necessary to determine the specific microbial succession that occurs during the fermentation of these and similar fermented food products. The chemical and physical characteristics of the fermentative process and the successive changes which occur during fermentation should be determined. Such studies will provide the data necessary for optimizing these food fermentations to produce better products of improved nutritional quality.

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